

## PHYSIOLOGY OF FREEZING RESISTANCE IN THE GENUS *Phaseolus*

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### Introduction

Late spring and early fall frosts are a major constraint to dry bean (*Phaseolus vulgaris* L.) production on the northern prairies. Common bean is sensitive to light frost (Levitt, 1980) and is killed at the moment of ice formation (-2°C). A late spring frost results in seedling death while early fall frost results in poor seed yield and quality. Due to the risk of late spring frost, dry bean is seeded usually in late May. Delayed seeding however, increases the risk of late season drought stress.

Buhrow (1980, 1983) reported frost resistance of tertiary gene pool of common bean in the field. Balasubramanian et al. (2000) reported that both tolerance and avoidance mechanisms of freezing resistance are observed in the tertiary gene pool, although avoidance is the primary mechanism of frost survival. Ice nucleators are ubiquitous, and reliability of supercooling as a viable frost survival mechanism in common bean is not known. Acclimation of plants to low non-freezing temperatures enhances subsequent freezing tolerance. Field screening of frost resistance of *Phaseolus* species will enable breeders to decide on appropriate parents and breeding strategies to introgress this trait into bean cultivars. The **objectives** of this study were to investigate i) the ability of common bean and four species in its tertiary gene pool to acclimate as a means of increasing freezing tolerance, and ii) freezing resistance of *Phaseolus* species in the field.

### Materials and Methods

CDC Nighthawk, two primary gene pool species *P. vulgaris* var. *mexicanus* (G11031, 2270 m – Sierra Madre) and *P. vulgaris* var. *aborigineus* (G23457, 2940 m; G23559, 2900 m; or G23454D, 2460 m – Andes), and four tertiary gene pool species *P. filiformis* (unknown), *P. angustissimus* (PI535272), *P. ritensis* (PI494138) and *P. acutifolius* var. *tenuifolius* (PI535248) were included. Seeds were from a uniform environment. With the exception of CDC Nighthawk, all other seeds were scarified by nicking the seed coat.

**1. Acclimation and Freezing Tolerance. Acclimation in the Natural Environment:** Accessions were grown in 4" pots filled with Rediearth at 23/18°C (8 h/16 h) with a 16 h photoperiod in growth chamber. The low temperature (18°C) overlapped both light and dark photoperiods. At 21 days after seeding, plants were in the V3 growth stage and were moved outdoors (April 28, 2000) for 14 days. Air temperature at plant height was monitored. Freezing dates were selected at 7 days intervals: 28 April (Control), 5 May and 12 May. Control plants were not moved outdoors. Freezing tolerance of plants was evaluated as follows: Four plants per accession were randomized in plastic trays. Four such trays were prepared and placed in the dark in a controlled environment chamber set at 0°C. Plants were sprayed with water kept at the chamber temperature. Air temperature was decreased to -2°C and subsequently decreased at a linear rate of 1°C h<sup>-1</sup> up to -5°C. Plants were removed at hourly intervals and placed in a chamber at 4°C. After 12 h at 4°C, the LT<sub>50</sub>K (lethal temperature at which 50% of the population is killed) was determined as the first sub-zero temperature at which two seedlings froze. Plants were then moved to a chamber at 23/18°C for 14 days. The LT<sub>50</sub>G (lethal temperature at which 50% of the population re-grew) was determined as the lowest sub-zero temperature at which two seedlings showed re-growth. Plants that escaped freezing injury were not considered for LT<sub>50</sub>K and LT<sub>50</sub>G determination.

**Acclimation in the Controlled Environment Chamber:** Accessions/cultivars were seeded on July 5, 2000 and grown outdoors for 26 days. Accessions were in the V3 or V4 growth stage and were moved to a chamber maintained at 7/5°C (PPFD  $\cong$  50  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>). The chamber was maintained at 7/5°C for 3 days and then decreased to 5/2°C and 2/0°C at three days interval. At the end of each temperature regime, freezing tolerance of plants was evaluated as described above. Control plants were subjected to 7/5°C.

Data were subjected to the analysis of variance appropriate to a split plot design with two replicates. Freezing dates were main plots and accessions/cultivar were sub plots, and both were considered as fixed effects.

**2. Freezing Resistance in the Field.** All accession/cultivar were seeded into Jiffy peat pellets and grown at 23/18°C (8 h/16 h) with a 16 h photoperiod for 15 days. One hundred seedlings per accession per replication were hand-transplanted in the field on 10<sup>th</sup> or 29<sup>th</sup> Aug., 2000. Two replicates per accession per transplanting date were used. Transplants were watered every day until establishment. Seedlings that survived transplant shock were counted. At the incidence of the first fall frost on Sept. 23, seedlings were in the V4 or R5 growth stage for those transplanted on 10 Aug., and were at the V3 growth stage for those transplanted on 29 Aug. Air temperature at plant height was monitored. Percentage survival on the 2<sup>nd</sup>, 7<sup>th</sup> and 14<sup>th</sup> day after the first fall frost was determined. Data were subjected to chi-square tests of independence of proportions to determine if the percentage survival/re-growth of seedlings is independent i) of its growth stage and ii) of the species.

### Results and Discussion

**1. Acclimation and Freezing Tolerance.** Freezing dates were not significantly different for LT<sub>50</sub>K and LT<sub>50</sub>D when

plants were acclimated under natural or controlled environment conditions (data not presented), indicating no significant increase in freezing tolerance (little or no acclimation). Seven days after plants were transferred outside (May 5), the  $LT_{50}K$  mean decreased from  $-1.9^{\circ}C$  to  $-3.1^{\circ}C$ . The decrease was not significant, probably due to fewer degrees of freedom for replication x freezing date interaction (d.f. = 2), which is the appropriate error to test the significance of freezing date. In the case of acclimation in controlled environment conditions, although the maximum and minimum air temperatures were controlled efficiently, no increase in freezing tolerance was observed across freezing dates for  $LT_{50}K$  and  $LT_{50}D$  (data not presented).

*Phaseolus* species differed significantly for  $LT_{50}K$  and  $LT_{50}D$  under both natural and controlled environment conditions. Three tertiary gene pool species *P. filiformis*, *P. angustissimus* and *P. ritensis*, in general were more freezing tolerant than the three primary gene pool species *P. vulgaris*, *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus*. *P. acutifolius* var. *tenuifolius* was either intermediate in response between the primary and tertiary gene pool or responded similarly to that of the primary gene pool.

When above ground shoot froze to death, growth from the cotyledonary nodes was observed for some seedlings in *P. angustissimus* and *P. ritensis*. Both have hypogeal germination. In *P. filiformis*, cotyledonary nodes are positioned at the soil surface and re-growth was observed in few seedlings.

When plants in 4" pots were subjected to a freeze test and observed under infrared thermography, the Rediearth froze at a chamber temperature of  $-4^{\circ}C$ . It is quite possible that seedling deaths in species with a lower  $LT_{50}K$ , and lack of re-growth from cotyledonary nodes in species with hypogeal germination were partly influenced by an earlier freezing of the Rediearth which in turn initiated nucleation of the seedlings. This however is uncommon in the field due to higher buffering capacity of soil.

**2. Freezing Resistance in the Field.** The first fall frost in Saskatoon in 2000 was on Sept. 23, and a subsequent frost occurred on Sept. 24, when the air temperatures at seedling height were  $-4.2^{\circ}C$  and  $-4.9^{\circ}C$ , respectively. Air temperature remained below  $-2^{\circ}C$  for 3 h on Sept. 23 and for 5.5 h on Sept. 24.

Primary gene pool species *Phaseolus vulgaris* cv. CDC Nighthawk, *P. vulgaris* var. *mexicanus* and *P. vulgaris* var. *aborigineus* were killed by the first fall frost regardless of growth stage of the seedling. These species showed no signs of recovery or re-growth for up to 7 days after the first fall frost, although frost did not occur during the above period, and air temperatures  $> 20^{\circ}C$  were recorded during the same period. This indicates the extreme susceptibility of the cultivated common bean and its primary gene pool to frost. The chi-square tests of independence of proportions indicated that among the tertiary gene pool species, with the exception of *P. filiformis* and *P. acutifolius* var. *tenuifolius*, seedling survival (on the 2<sup>nd</sup> day after frost) and/or re-growth (on the 7<sup>th</sup> day after frost) in *P. angustissimus* and *P. ritensis* were dependent on the growth stage of the species. In both *P. angustissimus* and *P. ritensis*, younger seedlings (second transplanting date) survived frost better than the relatively older seedlings (first transplanting date).

Chi-square tests of independence of proportions indicated that plant survival and re-growth on the 2<sup>nd</sup> and 7<sup>th</sup> day after the first fall frost were dependent on the *Phaseolus* species. On the 2<sup>nd</sup> day after the first fall frost, *P. angustissimus* had the highest percentage survival (74%) followed by *P. filiformis* (8%). Absence of frost between Sept. 24 and Oct. 2 coupled with air temperatures  $> 20^{\circ}C$  during the same period enabled surviving plants to re-grow. In *P. angustissimus*, increased number of surviving seedlings (76%) on the 7<sup>th</sup> day compared to the 2<sup>nd</sup> day after the first fall frost was due to growth of axillary shoots in seedlings with damaged terminal buds or due to growth from seedlings in which the stem survived the frost. In *P. filiformis*, seedlings either died to the ground and then produced new shoots from the cotyledonary nodes or survived the frost intact and had 16% seedling survival on the 7<sup>th</sup> day after the first fall frost. Starting Oct. 3, air temperature dropped to  $-10.2^{\circ}C$ , resulting in seedling death.

### Conclusions

Little or no acclimation was observed in *Phaseolus* species in response to low temperatures. Freezing of Rediearth soilless mix ahead of seedlings may have prevented us from studying the effect of acclimation, particularly in the tertiary gene pool. Freezing resistance of *P. angustissimus* in the field is promising. Introgression of freezing resistance into common bean genotypes may enable early to mid May seeding of dry bean on the Canadian prairies. This could further expand the geographic distribution of bean crop, possibly to higher altitudes in the tropics.

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### References

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